Short Communication

The behavioral and physiological effects of dog appeasing pheromone upon canine behavior during separation from the owner

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Abstract

Behavioral problems in the domestic dog *(Canis familiaris)* increase the likelihood of the dog being rehomed or relinquished to a rescue shelter. Problem behaviors that result in relinquishment include unwanted elimination, destructive behavior and excessive vocalization when owners are absent. Dog Appeasing Pheromone (DAP) is currently marketed via veterinarians as a stress relief product and purported to help dogs cope in stressful situations and as a potential solution to reduce anxiety. This study aimed to investigate if a DAP diffuser affected behavioral and physiological stress parameters in 10 dogs in a laboratory environment. A repeated measures design with and without the use of DAP, and in the presence and absence of the owner was used. Behavioral responses, such as barking, passive behavior, scratching, whining, oriented behavior, exploration and locomotion, were recorded in real time and video recorded using a focal instantaneous sampling technique. In order to control for potential bias, 10% of the videos were scored using a second blinded scorer to assess inter-rater reliability. Heart rate (HR), heart rate variability (HRV) using Standard Deviation of Normal to Normal beats (SDNN), eye temperature and ear temperature (°C) were also collected to assess dogs' physiological state. When dogs were separated from their owner, there was a significant increase in oriented behavior during both the DAP and without DAP application trial phase suggesting arousal due to owner absence rather than any discernible effect of DAP. A significant increase was recorded in core eye temperature when the owner was absent and the DAP diffuser was not switched on however, eye temperature also increased when the owners were present after the DAP condition suggesting that it may be the owner's presence and the dogs arousal levels that affect core eye temperature rather than any effect of DAP. There was no significant effect of DAP on HR or ear temperature. Overall, our results suggest that the application of a DAP diffuser did not markedly influence the behavior, heart rate, eye or ear temperature of dogs. Further investigation using a greater sample size and the use of further physiological stress indicators is recommended in order to further explore the potential application of DAP as a stress relief product for dogs.

Keywords: Dog Appeasing Pheromones; DAP Diffuser; Dog Behavior; Heart Rate; Ear and Eye Temperature

Introduction:

Dogs are commonly kept as pets, with 9 million dogs recorded as companion animals in the United Kingdom (UK) (PFMA, 2019) and over 76 million in the United States of America (USA) (AVMA, 2019). Work and lifestyle commitments for owners can often result in dogs being left at home for extended periods of time (Rehn and Keeling, 2011). Most dog owners work full time (Rehn and Keeling, 2011), with 73% of Swedish dog owners reporting they leave their dog at home during working hours (Norling and Keeling, 2010) and 39% of British owners leaving their dogs alone for at least seven hours (RSPCA, 2019). Dogs hospitalized in veterinary clinics can also result in separation from their owner (Kim et al., 2010). These absences can lead to behavioral conditions such as separation anxiety, one definition being distress caused when left or separated from a key person (Herron et al., 2014). Separation anxiety is prevalent in dogs (Dinwoodie et al., 2019; Tiira et al., 2016) and can result in problem behaviors such as inappropriate elimination, destructive behavior and distress vocalizations which occur when an owner is absent or perceived as absent, including when the owner is at home but the dog does not have access to them (Landsberg et al., 2013; Sherman and Mills, 2008; Ogata et al., 2016). Problem behaviors are a leading cause of dogs being rehomed or euthanized and can result in a loss of the human-animal bond (Sherman and Mills, 2008; Hargrave, 2014; Hewson, 2014), subsequently compromising welfare in dogs (Kim et al., 2010).

One approach which has been suggested to be efficacious in mediating the distress caused by separation of dogs from their owners is the use of Dog Appeasing Pheromone (DAP) (Adaptil, 2020). Dog Appeasing Pheromone is a commercially available product, which is advertised by the manufacturer to promote calm behavior in dogs (Adaptil, 2020). Dog Appeasing Pheromone is a combination of fatty acids synthetically created to replicate the pheromones released by a bitch during nursing to reassure young (Riemer, 2020). It can be dispersed via collar, diffuser, spray or tablet (Adaptil, 2020) and has been reported to reduce behavioral signs of anxiety in dogs in environments such as kennels (Amaya et al., 2020), veterinary facilities (Kim et al., 2010) and in the home environment when separated from the owner (Gaultier et al., 2008). However, the efficacy of DAP is difficult to gauge as there are a number of methodological issues inherent in the field. Poor inclusion criteria, unclear randomization methods and non-reporting of dogs with treatment failure have been commonly found in studies that tested the efficacy of DAP for the treatment of undesirable behavior in dogs (Frank et al., 2010).

To date, research has largely reported behavioral responses of dogs in response to DAP however physiological stress responses are not widely reported in DAP studies. Physiological parameters have been successfully used within other behavior studies to assess fear and anxiety in dogs (e.g. Brugarolas et al., 2015; Mariti et al., 2018). As behavioral signs can be non-specific and are often context based (Horwitz and Pike, 2014), behavioral states are inherently difficult to interpret. The inclusion of physiological stress responses is important for future studies investigating the efficacy of DAP to assess inner states which are often reflected in alterations at the behavioral level (Broach and Dunham, 2016; Grigg and Piehler, 2015). Physiological measures of stress include the use of heart rate variability (HRV) which is considered a useful tool for indicating fluctuations in the autonomic system that are indicative of stress responses (Brugarolas et al., 2015) and is unaffected by posture (Maros et al., 2008). Therefore, HRV is useful when paired with behavioral observations which may involve postural changes (Travain et al., 2016). Infrared thermography (IRT) is non-invasive and can be used to measure changes in core eye and ear temperature (Travain et al., 2015; Riemer et al., 2016) and can be paired with HRV as an indicator of psychological stress (Squibb et al., 2018). Heart rate and IRT measures offer supplementary physiological data to further support behavioral indicators of stress and can be used to assess stress response in dogs subjected to DAP application.

The aim of this study was to determine whether a DAP diffuser reduced behavioral and physiological stress responses in dogs when separated from their owners. As DAP is widely used in the clinical setting, the results are applicable to canine welfare and veterinary industries to inform clinical practice in relation to the efficacy of synthetic pheromones in reducing stress behaviors of dogs. They can also be used to assess whether DAPs are a worthwhile investment in reducing behavioral and physiological indicators of stress.

Materials and Methods

Subjects and Study Site

Participants were recruited over a two week period via social media (Facebook and Twitter) and encouraged to forward details of the study to interested parties. Whilst social media allows rapid dissemination of information, it is also possible that this method introduced demographic selection bias by recruiting younger and more internet active participants. To participate, dogs were required to be over 12 months of age, could be any breed or sex and did not demonstrate aggression towards strangers. Owners were asked whether their dog was clinically diagnosed with separation anxiety by a veterinarian or had any physical or behavioral disorders. None of the dogs were clinically diagnosed with separation anxiety or reported to have any physical or behavioral disorders. To address any welfare concerns, dogs were excluded from the study if they showed a high stress response during the experimental procedure (e.g. hyper-salivating, excessive panting). No dogs were withdrawn from the current study.

Ten dogs, 7 males (all neutered) and 3 females (2 spayed, 1 entire) of variable ages (8.1±4.1 years) were used in this study. Two of the dogs were cross breeds, other breeds included Jack

Russell Terriers (n=3), Beagles (n=2) and one of each of the following breeds; Springer Spaniel, Cocker Spaniel and Labrador Retriever. All dogs were recruited from a family home environment.

The study took place in a laboratory (7.3m x 6.4m) at Suffolk One Sixth Form centre, Ipswich. The laboratory temperature was set at 20°C±2. The laboratory contained no furnishings except for four diffusers (which were either switched on to emit DAP or left switched off depending on the condition). The diffusers were plugged into electrical sockets close to the corner of each of the four corners of the laboratory. The laboratory contained a glass door with a silver reflective one way mirror window film (T60-EV, Funime; EKA Home, China) which was used to enable real time behavioral observations by the experimenter, whilst maintaining visual separation from the dog. Behavior was scored real time by the experimenter and also video recorded to assess scoring reliability.

Procedure

A blinded, placebo-controlled, repeated measures design was deployed in order to account for individual variation in coping styles, sex, size and experience effects and any potential observer bias in scoring subjective behaviors. Activation of diffusers was completed by an independent party so the primary researcher was unaware of the conditions when scoring blinded behavioral videos (e.g. DAP, no DAP) to minimise bias. Owners and a second scorer for the behavioral videos were also unaware of conditions.

Dogs visited the study site on two occasions (approximately two days apart and at different times between 09.00h and 17.00h) and were exposed to a 45 minute procedure on each visit. The control condition consisted of owners placing the dog alone in the laboratory and going to a room approximately 10m from the laboratory for 5 minutes. Condition A (baseline) then began when the owner returned to the laboratory and stayed with the dog for 5 minutes. Condition B (trial) then began with the owner leaving again so the dog was alone in the laboratory for 30 minutes, with either DAP switched on or DAP switched off. Condition C (reunite) then began when the owner was reunited with the dog in the laboratory where they stayed with them for 5 minutes. The owners then removed their dog from the laboratory. Where owners were unavailable to participate in the trials, a familiar person was used instead. A familiar person (known to the dog and who engaged with the dog on a regular basis) was considered a suitable alternative to the owner as Riemer et al., (2016) observed no significant difference between dog responses when owners and strangers returned to the dog.

Dog Appeasing Pheromone was the condition which was presented first, with DAP being switched off on the second visit. In the DAP switched on condition, the diffuser was switched on prior to the focal dog entering the room for the trial phase (condition B). The conditions were not counterbalanced as it was not feasible due to the constraints of access to the facilities and the time period available however, to ensure the behaviors observed related to DAP, a one day "washout period" was instigated after the DAP switched on condition to allow the product to dissipate fully from the room and was aided by a built-in air conditioning system (EU200, Flaktwoods; Colchester, UK) which was switched on overnight. Four 48ml DAP refill diffusers (Adaptil; Ceva Santé Animale) were plugged into electrical sockets in each of the four corners of the laboratory to ensure even dispersal in to the surrounding environment. Each refill contained 2% DAP mixed in Isoparaffinic Hydrocarbon with each diffuser being replaced with a new refill for each subject.

Behavioral measures

The dogs' behavior was filmed throughout the 45 minute procedure using a GoPro video camera set at 60fps medium field of vision (GoPro Hero 3, GoPro Inc; San Mateo, California).

The video camera was located at the front of the laboratory on the work surface to enable full view of the laboratory. The observer was screened behind the mirror door at all times in order to reduce effects on the dogs' behavior. Focal instantaneous observations every 30 seconds were conducted, with behaviors recorded as present or absent using an adapted ethogram which included stress-related behaviors (Table 1). Video footage was analyzed at a later date to assess rater reliability.

Table 1

Ethogram providing definition of behaviors sampled in the DAP study (adapted from Beerda et al., 1999; Tod et al., 2005; Palestrini et al., 2010; Cannas et al., 2014).

Behavior	Definition
Exploration	Motor activity directed toward physical aspects of the environment, including sniffing and gentle licking
Locomotion	Walking or running around without exploring environment
*Hyper salivating	Excess visible drool around the mouth
Passive behavior	Lying down with head on ground without any obvious orientation toward physical or social environment
Orientated behavior	Sitting, standing or lying down (without head on the ground). Obvious orientation to the physical or social environment including sniffing, close or distant visual inspection
*Scratching environment	All handling with the forelimbs resulting in physical contact with the doors or walls, including jumping up
Oral behavior	Any vigorous behavior directed toward the environment using the mouth
*Panting	Mouth opens with tongue extended accompanied with rapid breathing and

	expansion/contraction of chest
Grooming	Action of cleaning the body surface by licking, nibbling, picking, rubbing, scratching towards the animal's own body
*Barking	"Rough" sound often repeated in quick succession
*Whining	Whining
*Howling	Howling
*Trembling	Shaking movements of the body/head
Paw up	A front limb raised
Circling	Movement of the dog in circles
*Yawning	Mouth opens wide for a period of a few seconds and then closes
*Lip licking	Part of the tongue is shown and moved along the upper lip
Elimination urine	Elimination of urine
Elimination faeces	Elimination of faeces

*indicates stress-related behaviors

Behavioral data for 10% of the videos were also scored using a second blinded scorer (Cannas et al., 2014) to ensure inter-rater reliability using Spearman correlations and Cohen's kappa. There was a strong correlation between experimenter and second naïve independent rater when scoring behavioral data (Test 1: $r_s(14) = 0.811$, $P<0.001$, Test 2: $r_s(14) = 0.858$, $P<0.001$, Test 3: $r_s(14) = 0.756$, $P<0.001$). Inter-rater reliability was found to show between fair and moderate agreement between the experimenter and the independent, blinded second scorer and behavior scores (Cohen's κ: Test 1: $\kappa = 0.289$, 95% CI 0.142 to 0.436, $P<0.01$, Cohen's κ: Test 2: $\kappa = 0.471$, 95% CI 0.210 to 0.732, $P<0.001$, Cohen's κ: Test 3: $\kappa = 0.279$, 95% CI 0.101 to 0.457, $P<0.05$) (Altman, 1991; Landis and Koch, 1977). Both the primary researcher and independent scorer were unaware of conditions allocated at the time of scoring. Behavioral observations scored by the experimenter were used during data analysis.

Physiological measures

Heart rate variability (HRV) was recorded throughout all conditions of the 45 minute procedure with standard deviation of normal to normal R-R intervals (SDNN) recorded. A RS800CX Polar heart rate monitor (Polar Electro UK Ltd, Warwick, UK) with elasticated strap was fitted to the dog prior to entering the laboratory (Travain et al., 2016; Wormald et al., 2017). 3M Vetrap (3M Vetrap, 3M animal Care Products, St-Paul MN, USA) was fitted over the strap to ensure the monitor was secure and to maintain conduction. Warm water was applied to the dogs coat until wet through to the skin and was applied behind the legs from the sternum up to and level with the point of the shoulder to aid conduction. Ultrasound gel was also applied liberally to the electrode extensions of the sensor to improve conduction (Jonckheer-Sheehy et al., 2012; King et al., 2014). The sensor was placed in the left axillary region and was fitted snuggly (two fingers under the strap). The transmitter and receiver were checked to be connected prior to entering the room. Dogs were considered habituated to wearing the monitor when moving forward and not scratching or biting it (Rehn and Keeling, 2011).

Infrared Thermography (IRT) was used as a non-invasive stress assessment method to record core eye temperature (Stewart et al., 2005; Travain et al., 2015) and ear pinnae temperature (Riemer et al., 2016) using a portable IRT camera (FLIR One iOS plug-in Thermal Imaging Camera, USA, FLIR™). Five thermal image readings took place between conditions e.g. immediately before and after the control condition and also immediately post conditions A, B and C. Thermographic measurements measured temperature (°C) in the lacrimal caruncle of the eye, since this has been shown to represent the core body temperature in dogs (Travain et al., 2015). Measurements of the left or right eye temperature were randomly assigned. Ear pinnae temperature (°C) spots were added to the same image, and placed from tip to base to form a triangle to measure dynamic changes in ear temperature (Riemer et al., 2016). Dogs were gently restrained by their collar. Images were taken at 1m from the subject and at an angle of 90° (Travain et al., 2015). All photos were taken within the laboratory room. All the images were analyzed using thermal imaging analysis software (FLIR Tools 1.8.2(46)).

Data analysis

The total frequency each dog was observed performing each behavior was summed, providing an overall frequency count per dog per behavior during each condition with DAP application or without DAP switched on. Where behaviors were exhibited at very low levels (mean occurrence < 1), they were omitted from the analysis as statistical analyses are not robust at such low levels. Mean heart rate, heart rate variability, eye and ear temperature were analyzed per dog, per condition with DAP application or without DAP switched on. The significance level was set a priori at $P<0.05$ and all statistical analyses were performed using SPSS v.26 (IBM SPSS Statistics, 2019). Friedman ANOVA tests were used to determine whether DAP application significantly affected the dogs' behavior across the conditions. Where these tests found significant differences, post hoc Wilcoxon's signed-rank tests were used to determine where differences existed between conditions. Two-way Repeated Measures ANOVA tests were used to determine if there was a statistically significant interaction effect between the factors of condition and pheromone on the dependent variables measured (e.g. heart rate, heart rate variability, eye and ear temperature), across all conditions observed. The Mauchly sphericity test was used for within-subject effect. For cases that did not meet the sphericity condition ($P<0.05$), the Greenhouse– Geisser correction was applied. Where these tests found significant differences, post hoc pairwise comparisons were used to identify where differences existed between factors. To control for potential type 1 errors due to the repeated measures designs, a Bonferroni adjustment was applied for all post-hoc analyses with the revised alpha set at $P<0.01$. Inter-rater reliability was assessed using Spearman correlations and Cohen's κappa.

Results

Effect of DAP application on dog behavior

There was a significant effect of DAP application on barking behavior ($\chi^2(7, n = 10) = 29.556$, $P<0.001$), passive behavior ($\chi^2(7, n = 10) = 15.626$, $P<0.05$), scratching behavior ($\chi^2(7, n = 10) = 19.948$, $P<0.01$) whining behavior ($\chi^2(7, n = 10) = 37.823$, $P<0.001$) and oriented behavior ($\chi^2(7, n = 10) = 42.742$, $P<0.001$) with overall higher levels of barking and oriented behaviors being exhibited in the DAP application condition and overall higher levels of passive, scratching and whining behaviors being exhibited in the without DAP condition (Supplementary File 1). However after the Bonferroni correction (adjusted alpha: $P<0.01$), there was only a significant difference in oriented behavior between conditions (without DAP Reunite and without DAP Trial: $P=0.001$; without DAP Reunite and DAP application Trial: $P<0.001$; DAP application Baseline and without DAP Trial: $P=0.007$; DAP application Baseline and DAP application Trial: $P=0.001$; without DAP Baseline and without DAP Trial: $P=0.009$; without DAP Baseline and DAP application Trial: $P=0.002$) (Supplementary file 2).

Median frequency of oriented behavior was higher during conditions when the owner was not present and DAP was not switched on (trial: 43.5) than when the owner was present and DAP was not switched on (reunite: 4.0) (Figure 1). Oriented behavior was also higher during conditions when the owner was not present and DAP switched on (trial: 56.0) than when the owner was present and DAP not switched on (reunite: 4.0). Oriented behavior was higher during conditions when the owner was not present and DAP was not switched on (trial: 43.5) than when the owner was present (baseline: 4.0). Oriented behavior was higher during conditions when the owner was not present and DAP was not switched on (trial: 43.5) than when the owner was present and DAP was not switched on (baseline: 5.0). Oriented behavior was higher during conditions when the owner was not present and DAP was switched on (trial: 56.0) than when the owner was present and DAP was not switched on (baseline: 5.0). Oriented behavior was also higher during conditions when the owner was not present and DAP switched on (trial: 56.0) than when the owner was present (baseline: 4.0).

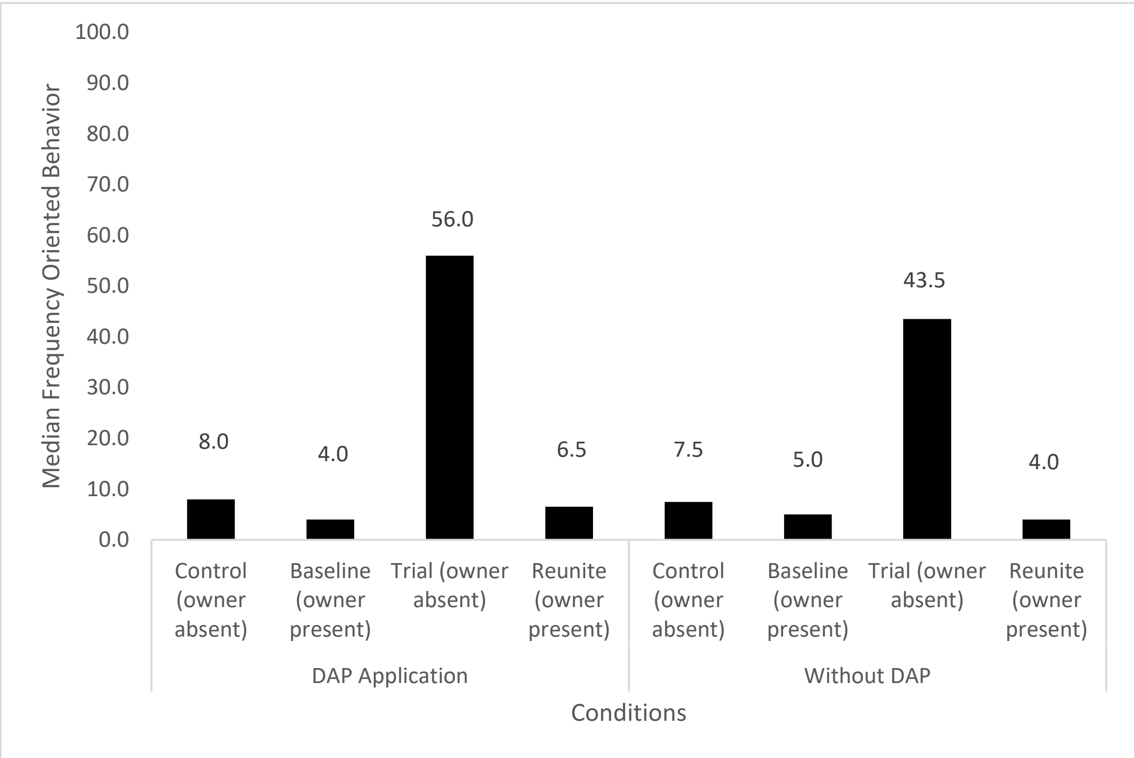

Figure 1: Median frequency of oriented behavior and conditions with DAP application and without DAP during the trial condition.

There was no significant effect of DAP application on exploration behavior ($\chi^2(7, n = 10) = 3.467$, $P>0.05$) and locomotion behavior ($\chi^2(7, n = 10) = 8.023$, $P>0.05$). Hyper-salivating behavior, oral behavior, panting, grooming, howling, trembling, paw up and circling occurred at very low levels and therefore these behaviors were omitted from the statistical analyses.

Effect of DAP application on dog heart rate

There was no significant main effect for DAP ($F(1,9) = 1.196$, $P>0.05$) or conditions ($F(3,24) = 2.441$, $P>0.05$) for mean heart rate. There were no significant interactions between the variables (e.g. interaction effect between the factors of condition and pheromone on the dependent variable heart rate) ($F(3,24) = 0.351$, $P>0.05$). There was no significant main effect for DAP ($F(1,9) = 1.679$, $P>0.05$) or conditions ($F(3,24) = 0.236$, $P>0.05$) for heart rate variability (SDNN). There were no significant interactions between the variables (e.g. interaction effect between the factors of condition and pheromone on the dependent variable heart rate variability) ($F(3,24) = 1.200$, $P>0.05$).

Effect of DAP application on dog eye temperature

There was no significant main effect for DAP ($F(1, 9) = 0.033$, $P>0.05$) however, there was a significant main effect for the conditions baseline and reunite ($F(4, 32) = 0.023$, $P<0.01$). Prior to the Bonferroni adjustment there was a significant difference between the conditions (start and baseline: $P=0.017$; start and reunite: $P=0.030$), however after the Bonferroni adjustment there was only a significant difference between the baseline and trial conditions ($P=0.005$).

Mean eye temperature was higher during conditions when the owner was absent and DAP was not switched on (trial 34.92°C) than when owners were present prior to the DAP application (baseline 29.50°C) (Figure 2). There was no interaction between the variables (e.g. interaction effect between the factors of condition and pheromone on the dependent variable eye temperature) ($F(4, 32) = 0.345$, $P>0.05$).

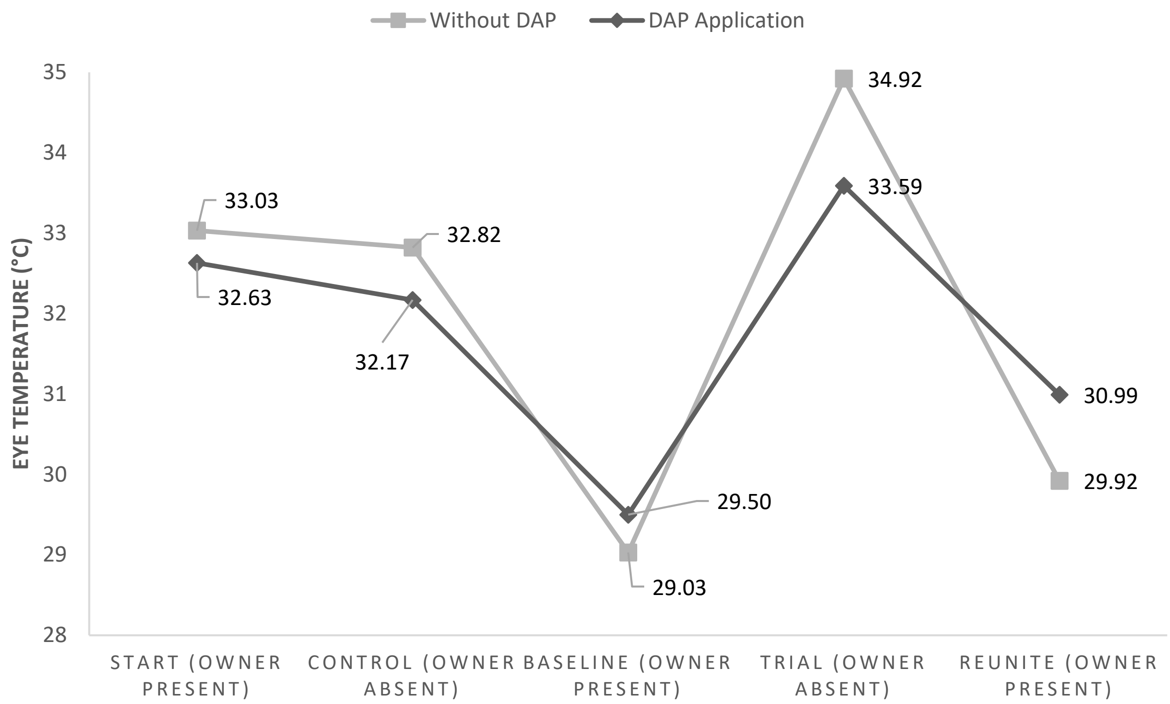

Figure 2: Mean Eye Temperature (°C) across conditions with DAP application and without DAP during the trial condition

Effect of DAP application on dog ear temperature

There was no significant main effect for DAP ($F(1, 9) = 0.747$, $P>0.05$) however, there was a significant main effect for the conditions start, baseline and trial ($F(4, 36) = 5.147$, $P<0.05$). Prior to the Bonferroni adjustment there was a significant difference between the conditions (start and baseline: $P=0.018$; baseline and trial: $P=0.031$), although after the Bonferroni adjustment ear pinnae temperature did not differ significantly across conditions ($P>0.01$). There was no interaction between the variables (e.g. interaction effect between the factors of condition and pheromone on the dependent variable ear temperature) ($F(4, 36) = 0.227$, $P>0.05$).

Discussion

The findings from this study suggest that DAP does not have a marked influence upon the behavior of dogs in a laboratory environment. When dogs were separated from their owner, there was an increase in oriented behavior during both the DAP application trial phase and without DAP suggesting that exposure to DAP does not significantly influence oriented behavior in the dogs observed in this study but rather the absence of the owner. There was no effect of DAP found for other behavioral measures. Eye temperature overall was lower when owners were absent and DAP was switched on however, eye temperature increased when the owners were present after the DAP application suggesting that it may be the owner's presence that affects eye temperature rather than any discernible effect of DAP. These findings cast doubts on the efficacy of DAP use as an adjunct therapy to relieve stress-related behavior and physiological responses to stress in dogs.

Our data did not show a reduction in stress-related behavior; it is possible that DAP diffusers may simply not have been effective in this context. For example, the laboratory environment may have been too stressful for DAP to have a marked effect.Environments that are both uncontrollable and unpredictable can be a stressor for dogs (Tuber et al., 1999). Given that the owners left their dogs on multiple occasions throughout the study, the stress incurred may have been too great for DAP to have an effect during the trial condition. In addition, the process of pheromone processing is not entirely understood (Broach and Dunham, 2016), and it may be possible that pheromonal analog products produce only mild effects (Hermiston et al., 2018).

The small sample size may have contributed to these findings. While further research repeating this study with a larger sample size would be of value, other explanations need to be considered for the lack of observable differences in behavior and eye temperature seen in this study. Changes in oriented behavior appear to relate to arousal levels during the presence or absence of the owner during the different conditions. This supports others who have found increased orientation towards the door during owner absence (e.g. Topál et al., 1998; Parthsarathy and Crowell-Davis, 2006; Schwab and Huber, 2006; Fallani et al., 2007; Palestrini et al., 2010). Schwab and Huber (2006) also found dogs have a larger variation in position, latency and proportion of response when the owner is absent. Dogs are also reported to differ in their behaviors in novel environments unless accompanied by their owner (Palmer and Custance, 2008). This could account for behavioral changes when the owner left the dog during the baseline and trial phases and also reunite stages when the owner returned to accompany their dog (for example, an increase in arousal when separated and reduced arousal and reduced oriented behavior when owner returned).

Limited control over access to the facilities and time constraints meant that counterbalancing the conditions and ensuring dogs visited the site at the same time was not possible in the current study. Whilst a wash out period was instigated to try to reduce potential confounding effects, learning may have occurred and non-randomising of order and time effects are potential confounding factors. As such, our results should be interpreted with caution when attempting to draw conclusions regarding the efficacy of DAPs. The DAP application condition was the first condition dogs were exposed to and as such the laboratory environment that the study took place in would have been considered a novel environment for the dogs. This may have instigated an increase in oriented behavior compared to the without DAP condition where dogs will have been more familiar with the environment which may have accounted for a decrease in oriented behavior during the without DAP condition. It has also been reported that responses can change in relation to the novelty of the environment, and are relative to where a response has been learnt (Braem and Mills, 2010). It may be that as the current study incorporated a short control period to account for the novel environment, that dogs did not habituate in the time given, and so the lack of significant differences in the majority of the physiological parameters indicated the novel environment was stressful throughout (Palmer and Custance, 2008). Further research is required using a larger sample size and evaluation of other indicators of stress in dogs (such as cortisol) in order to further explore the potential of DAP as a stress relief product for dogs.

In the current study, eye temperature increased when owners were absent. As the environment was novel and many dogs find owner separation stressful (Topál et al., 1998; Prato-Previde et al., 2003), there is strong support for the assumption that the dogs in the current study had increased eye temperature in response to stress caused from owner separation. Nonetheless, changes in eye temperature may simply reflect change in arousal rather than emotional valance (Travain et al., 2016) and therefore these results should be interpreted with caution. IRT is a relatively young field when used to assess stress responses in dogs. More studies are needed to investigate if there are any eye temperature changes in relation to canine stress responses, if this change in eye temperature is always in one direction and if this response is lateralised, which was not evaluated in the current study.

No significant difference was found in cardiac data or core ear temperature relating to owner presence and absence across conditions. Other studies have reported the absence of the owner did not have an effect on dogs' mean HR (e.g. Maros et al., 2008; Gasci et al., 2013). Our results also support other studies where variables such as HRV or cortisol were examined to evaluate the hypothalamic-pituitary-adrenal axis (HPA), and no significant effects of pheromone application were found (e.g. Berger et al., 2013; de Paula et al., 2019). However, large individual variation was found with regard to the HR and HRV by Maros et al., (2008). Future studies should therefore include larger sample sizes, to reduce the impact of individual variation. Future research is required to further validate IRT methodology such as obtaining correlations with heart rate, cortisol levels and behavior.

Conclusions

Our results indicate that the application of a DAP diffuser did not markedly influence the behavior, heart rate, eye or ear temperature of dogs in a laboratory environment. Increases in oriented behavior in both the DAP and without DAP conditions may indicate arousal due to owner absence. Moreover, it may be the owner's presence that affects changes in core eye temperature rather than any discernible effect of DAP. Although our results should be interpreted with caution, veterinary professionals should be cautious about recommending such products to clients until there is a stronger evidentiary basis supporting the use of DAP. Further investigation using a greater sample size, longer duration of DAP exposure and testing within the home environment would be of value. Use of physiological stress indicators, such as cortisol alongside behavioral indicators would also be beneficial. In addition, future research is required to further validate IRT methodology such as obtaining correlations with heart rate, cortisol levels and behavior.

Conflict of interest statement

The authors have no conflict of interests to declare. None of the authors of this paper have a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Ethical considerations

Approval for the study was not needed under the Animals Scientific Procedures Act 1986 or the European Union Directive 2010/63/European Union. The study abided by the guidelines of the Institutional Research Ethics Committee.

Authorship

The idea for the article was conceived by Lucy Webb and Jane Williams. The experiments were designed by Lucy Webb and Jane Williams. The experiments were performed by Lucy Webb. The data were analyzed by Lucy Webb and Sienna Taylor. The article was written by all the authors.

References

Adaptil, 2020. Adaptil. Available at: http://www.adaptil.com/uk. Accessed March 12, 2020.

Altman, D.G., 1991. Practical Statistics for Medical Research. Chapman and Hall, London.

Amaya, V., Paterson, M. and Phillips, C.J., 2020. Effects of Olfactory and Auditory Enrichment on the Behaviour of Shelter Dogs. *Animals*. 10(4), 581.

AVMA, 2019. US Pet Ownership Statistics. Available at: https://www.avma.org/resources-tools/reports-statistics/us-pet-ownership-statistics. Accessed 8 May 2020.

Beerda, B., Schilder, M., Bernadina, W., Van Hooff, J., De Vries, H., Mol, J., 1999. Chronic stress in dogs subjected to social and spatial restriction. II. Hormonal and immunological responses. Physiol. Behav. 66, 243e254.

Berger, J.M., Spier, S.J., Davies, R., Gardner, I.A., Leutenegger, C.M. and Bain, M., 2013. Behavioral and physiological responses of weaned foals treated with equine appeasing pheromone: A double-blinded, placebo-controlled, randomized trial. J. Vet. Behav. 8(4), 265-277.

Braem, M.D. and Mills, D.S., 2010. Factors affecting response of dogs to obedience instruction: A field and experimental study. Appl Anim. Behav. Sci. 125(1-2), 47-55.

Broach, D., Dunham, A.E., 2016. Evaluation of a pheromone collar on canine behaviors during transition from foster homes to a training kennel in juvenile Military Working Dogs. J. Vet. Behav. 14, 41-51.

Brugarolas, R., Latif, T., Dieffenderfer, J., Walker, K., Yuschak, S., Sherman, B.L., Roberts, D.L., Bozkurt, A., 2015. Wearable Heart Rate Sensor Systems for Wireless Canine Health Monitoring. IEEE Sens. J. 16(10), 3454-3464.

Cannas, S., Frank, D., Minero, M., Aspesi, A., Benedetti, R., Palestrini, C., 2014. Video analysis of dogs suffering from anxiety when left home alone and treated with clomipramine. J. Vet. Behav. 9(2), pp.50-57.

de Paula, R.A., Aleixo, A.S.C., da Silva, L.P., Grandi, M.C., Tsunemi, M.H., Lourenço, M.L.G. and Chiacchio, S.B., 2019. A test of the effects of the equine maternal pheromone on the clinical and ethological parameters of equines undergoing hoof trimming. J. Vet. Beh. 31, 28-35.

Fallani, G., Previde, E.P. and Valsecchi, P., 2007. Behavioral and physiological responses of guide dogs to a situation of emotional distress. Physio. Behav. 90(4), 648-655.

Frank, D., Beauchamp, G., Palestrini, C., 2010. Systematic review of the use of pheromones for treatment of undesirable behavior in cats and dogs. J. Am. Vet. Med. Assoc. 236, 1308e1316.

Gácsi, M., Maros, K., Sernkvist, S., Faragó, T. and Miklósi, Á., 2013. Human analogue safe haven effect of the owner: behavioural and heart rate response to stressful social stimuli in dogs. PLoS One. 8(3), e58475.

Gaultier, E., Bonnafous, L., Vienet-Lague, D., Falewee, C., Bougrat, L., Lafont-Lecuelle, C. and Pageat, P., 2008. Efficacy of dog-appeasing pheromone in reducing behaviours associated with fear of unfamiliar people and new surroundings in newly adopted puppies. Vet. Rec. 164(23), 708-714.

Grigg, E.K., Piehler, M., 2015. Influence of dog appeasing pheromone (DAP) on dogs housed in a long-term kennelling facility. Vet. Rec. 2(1), e000098.

Hargrave, C., 2014. Pheromonatherapy and animal behaviour : providing a place of greater safety. Comp. Anim. 19(2).

Hermiston, C., Montrose, V.T. and Taylor, S., 2018. The effects of dog-appeasing pheromone spray upon canine vocalizations and stress-related behaviors in a rescue shelter. J. Vet. Beh. 26, 11-16.

Herron, M.E., Lord, L.K. and Husseini, S.E., 2014. Effects of preadoption counseling on the prevention of separation anxiety in newly adopted shelter dogs. J.Vet. Behav. 9(1), 3-21.

Hewson, C., 2014. Evidence-based approaches to reducing in-patient stress – Part 2: Synthetic pheromone preparations. Vet. Nurs. J. 29(6), 204–206.

Horwitz, D.F., Pike, A.L., 2014. Common sense behavior modification: a guide for practitioners. Vet. Clin. N. Am. Small. 44(3), 401-426.

Jonckheer-Sheehy, V., Vinke, C., Ortolani, A., 2012. Validation of a Polar® human heart rate monitor for measuring heart rate and heart rate variability in adult dogs under stationary conditions. J. Vet. Behav. 7(4), 205-212.

Kim, Y.M., Lee, J.K., Abd el-Aty, A.M., Hwang, S.H., Lee, J.H., Lee, S.M., 2010. Efficacy of dog-appeasing pheromone (DAP) for ameliorating separation-related behavioral signs in hospitalized dogs. Can. Vet. 51(4), 380.

King, C., Buffington, L., Smith, T.J., Grandin, T., 2014. The effect of a pressure wrap (ThunderShirt®) on heart rate and behavior in canines diagnosed with anxiety disorder. J. Vet. Behav. 9(5), 215-221.

Landsberg, G., Hunthausen, W. and Ackerman, L., 2013. Behavior Problems of the Dog and Cat-E-Book. London: Elsevier Health Sciences (Accessed 02/12/2019)

Landis, J.R., Koch, G.G., 1977. The measurement of observer agreement for categorical data. Biometrics. 33, 159–174.

Mariti, C., Carlone, B., Protti, M., Diverio, S. and Gazzano, A., 2018. Effects of petting before a brief separation from the owner on dog behavior and physiology: A pilot study. J. Vet. Behav. 27, 41-46.

Maros, K., Dóka, A., Miklósi, Á., 2008. Behavioural correlation of heart rate changes in family dogs. Appl. Anim. Behav. Sci. 109(2-4), 329-341.

Norling, A.Y., Keeling, L., 2010. Owning a dog and working: A telephone survey of dog owners and employers in Sweden. Anthrozoös. 23(2), 157-171.

Palestrini, C., Minero, M., Cannas, S., Rossi, E., Frank, D., 2010. Video analysis of dogs with separation-related behaviors. Appl. Anim. Behav. Sci. 124(1-2), 61-67.

PFMA (2019). Pet Population. Available from. https://www.pfma.org.uk/pet-population-2019 Accessed 31st Dec 2019.

Palmer, R. and Custance, D., 2008. A counterbalanced version of Ainsworth's Strange Situation Procedure reveals secure-base effects in dog–human relationships. Appl Anim. Behav. Sci. 109(2-4), 306-319.

Parthasarathy, V. and Crowell-Davis, S.L., 2006. Relationship between attachment to owners and separation anxiety in pet dogs (Canis lupus familiaris). J. Vet. Behav. 1(3), 109-120.

Prato-Previde, E., Spiezio, C., Sabatini, F. and Custance, D.M., 2003. Is the dog-human relationship an attachment bond? An observational study using Ainsworth's strange situation. Behav. 140(2), 225-254.

Riemer, S., Assis, L., Pike, T.W., Mills, D.S., 2016. Dynamic changes in ear temperature in relation to separation distress in dogs. Physiol. Behav. 167, 86-91.

Riemer, S., 2020. Effectiveness of treatments for firework fears in dogs. J. Vet. Behav. 37, 61-70.

Rehn, T., Keeling, L., 2011. The effect of time left alone at home on dog welfare. Appl. Anim. Behav. Sci. 29(2-4), 129-135.

RSPCA, 2019. One fifth of dog owners believe it's okay to leave pets alone for more than 24 hours. Available at: https://www.rspca.org.uk/whatwedo/latest/news/details/-/articleName/fifth-of-dog-owners-believe-it-s-okay-to-leave-pets-alone-for-more-than-24-hours. Accessed 8 May 2020.

Schwab, C. and Huber, L., 2006. Obey or not obey? Dogs (Canis familiaris) behave differently in response to attentional states of their owners. J. Comp. Psychol. 120(3), 169.

Sherman, B.L., Mills, D.S., 2008. Canine Anxieties and Phobias: An Update on Separation Anxiety and Noise Aversions. Vet. Clin. N. Am. Small. 38(5), 1081–1106.

Squibb, K., Griffin, K., Favier, R., Ijichi, C., 2018. Poker Face: Discrepancies in behaviour and affective states in horses during stressful handling procedures. Appl. Anim. Behav. Sci. 202, 34-38.

Stewart, M., Webster, J.R., Schaefer, A.L., Cook, N.J., Scott, S.L., 2005. Infrared thermography as a non-invasive tool to study animal welfare. Anim. Welf. 14, 319–325

Topál, J., Miklósi, Á., Csányi, V. and Dóka, A., 1998. Attachment behavior in dogs (Canis familiaris): a new application of Ainsworth's (1969) Strange Situation Test. J. Comp. Physiol. 112(3), 219.

Tuber, D.S., Miller, D.D., Caris, K.A., Halter, R., Linden, F., Hennessy, M.B., 1999. Dogs in animal shelters: problems, suggestions, and needed expertise. Psychol. Sci. 10, 379e386.

Tod, E., Brander, D., Waran, N., 2005. Efficacy of dog appeasing pheromone in reducing stress and fear related behavior in shelter dogs. Appl. Anim. Behav. Sci. 93, 295e308.

Travain, T., Colombo, E., Heinzl, E., Bellucci, D., Prato Previde, E.,Valsecchi, P., 2015. Hot dogs: Thermography in the assessment of stress in dogs (Canis familiaris)—A pilot study. J. Vet. Behav. 10(1), 17-23.

Travain, T., Colombo, E.S., Grandi, L.C., Heinzl, E., Pelosi, A., Previde, E.P., Valsecchi, P., 2016. How good is this food? A study on dogs' emotional responses to a potentially pleasant event using infrared thermography. Physiol. Behav. 159, 80-87.

Wormald, D., Lawrence, A.J., Carter, G., Fisher, A.D., 2017. Reduced heart rate variability in pet dogs affected by anxiety-related behaviour problems. Physiol. Behav. 168, 122-127.